Mechanisms of bradykinin-mediated dilation in newborn piglet pulmonary conducting and resistance vessels

JUDY L. ASCHNER,1 THUY K. SMITH,1 NORA KOVACS,1 JOAQUIM M. B. PINHEIRO,2 AND MAMTA FULORIA1

1Department of Pediatrics, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157-1081; and 2Department of Pediatrics, Albany Medical College, Albany, New York 12208

Received 22 January 2002; accepted in final form 18 March 2002

Aschner, Judy L., Thuy K. Smith, Nora Kovacs, Joaquim M. B. Pinheiro, and Mamta Fuloria. Mechanisms of bradykinin-mediated dilation in newborn piglet pulmonary conducting and resistance vessels. Am J Physiol Lung Cell Mol Physiol 283: L373–L382, 2002.—Bradykinin (BK) is a potent dilator of the perinatal pulmonary circulation. We investigated segmental differences in BK-induced dilation in newborn pig large conducting pulmonary artery and vein rings and in pressurized pulmonary resistance arteries (PRA). In conducting pulmonary arteries and veins, BK-induced relaxation is abolished by endothelial disruption and by inhibition of nitric oxide (NO) synthase with nitro-L-arginine (L-NA). In PRA, two-thirds of the dilation response is L-NA insensitive. Charybdoxin plus apamin and depolarization with KCl abolish the L-NA-insensitive dilations, findings that implicate the release of endothelium-derived hyperpolarizing factor (EDHF). However, endothelium-disrupted PRA retain the ability to dilate to BK but not to ACh or A-23187. In endothelium-disrupted PRA, dilation was inhibited by charybdotoxin. Thus in PRA, BK elicits dilation by multiple and duplicative signaling pathways. Release of NO and EDHF contributes to the response in endothelium-intact PRA; in endothelium-disrupted PRA, dilation occurs by direct activation of vascular smooth muscle calcium-dependent potassium channels. Redundant signaling pathways mediating pulmonary dilation to BK may be required to assure a smooth transition to extrauterine life.

pulmonary resistance arteries; endothelium; nitric oxide; calcium-dependent potassium channels; endothelium-derived hyperpolarizing factor

isolated vessel segments are useful for studying mechanisms regulating vascular tone and reactivity. Most studies of pulmonary vascular responses have utilized large conducting vessels because of the technical ease of isolating relatively large vessels. However, large conducting arteries contribute little to pulmonary vascular resistance (PVR) (60). Intravascular measurements have shown that precapillary resistance is divided approximately equally between the small resistance arteries and arterioles (41). Small resistance arteries are defined as those vessels proximal to the arterioles with diameters <500 μm (41). As the principal site of increased PVR, these small arteries are a major determinant of organ blood pressure and blood flow distribution (57).

Previous studies have demonstrated marked heterogeneity in the responses of blood vessels to dilator and constrictor stimuli. Species-dependent, age-dependent, and organ-specific differences have been described (13, 38, 46, 49, 55). Even within a given vascular bed, segmental differences have been noted in the responses of arteries and resistance vessels and veins (1, 20, 26, 32, 37, 38, 55, 56). Although segmental differences in vasomotor reactivity are well documented, the mechanisms controlling local variations in the responses of vessels of varying size and origin are unknown. Different endothelium-dependent factors, vascular smooth muscle (VSM) membrane receptors or distal signaling processes may exist between large and small vessels.

In these studies we addressed the hypothesis that different signaling mechanisms mediate vasodilator responses in pulmonary resistance arteries (PRA; luminal diameter <300 μm) and in large conducting (>500 μm diameter) pulmonary arteries (PA), and pulmonary veins (PV). To test this hypothesis, we examined the responses of these three vessel segments from newborn pig lungs to the peptide bradykinin (BK).

BK is believed to play a physiological role in the circulatory changes that occur immediately after birth (7, 24, 39), although BK receptor blockade does not prevent the oxygen-mediated decline in PVR in fetal lambs (2). The concentration of kininogen, the precursor of BK, decreases at birth, and BK concentrations in newborn pig lungs to the peptide bradykinin (BK).
also show that the operative signaling mechanisms mediating dilation in response to BK in the pulmonary arterial and venous circulations differ from those regulating BK-mediated dilation in the pulmonary microcirculation.

METHODS

Drugs. The chemicals, dosages, and signaling pathways targeted in the investigations of BK-mediated dilation are listed in Table 1. Comments and caveats regarding drug specificity and sites of action are included in the accompanying legend.

Drug stock solutions were prepared as follows: 4-amino-pyridine (4-AP), acetylcholine (ACh), apamin (APA), BK, and sodium nitroprusside (SNP) were prepared as aqueous solutions. Precautions were taken to protect the SNP solution from light. A-23187, 17-octadecyenoic acid (17-ODYA), glibenclamide (Glib), and miconazole were dissolved in DMSO; 9, 11-dideoxy-11α, 9α-epoxy-methanoprostaglandin F2α (U-46619) was dissolved in ethanol; charybdoxin (CTX) was dissolved in 150 mM NaCl; indomethacin (Indo) was prepared in 250 mM Na2CO3, and nitro-l-arginine (l-NA) was dissolved in double-distilled H2O with drop-wise addition of HCl (1 M) until dissolved. High K+ solutions were prepared by isosmotic substitution of NaCl with KCl. In each case, the vehicle had no effect on the final pH of the buffer or on vascular reactivity of PRA or large vessel ring preparations. All drug concentrations are expressed as final molar concentrations in the organ bath or PRA arteriograph.

HOE-140 was a gift from Hoechst-Roussel Pharmaceuticals (Somerville, NJ). Calbiochem (San Diego, CA) was the source of U-46619. All other chemicals were purchased from Sigma (St. Louis, MO).

Animals. Newborn piglets, 1–4 days of age, were killed with a lethal intraperitoneal injection (75–100 mg/kg) of pentobarbital sodium. Heart and lungs were removed en bloc and stored in cold, oxygenated physiological bicarbonate solution containing 1.6 mM CaCl2 before study (1–24 h). All experimental protocols were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals and approved by the Animal Care and Use Committee of Wake Forest University School of Medicine. The Animal Resource Facilities of Wake Forest University School of Medicine are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Isolation of pulmonary conducting vessels for measurement of isometric tension. Under a dissecting microscope, third to fourth generation PA and PV with internal diameters of 500–1,000 μm were dissected from surrounding lung parenchyma and cleaned of excess connective tissue. Vascular rings, 3–5 mm in length, were mounted between two parallel triangular-shaped 28-gauge stainless steel wires (Arista Surgical, New York, NY) and placed in individual 16-ml tissue baths (Radnoti Glass, Monrovia, CA) filled with bicarbonate-based buffer with the following millimolar composition: 130 NaCl, 4.7 KCl, 1.17 MgSO4·7 H2O, 1.18 KH2PO4, 14.9 NaHCO3, 5.5 dextrose, 0.03 NaCa2EDTA, and 1.6 CaCl2·2 H2O. The buffer was maintained at 37°C and equilibrated with normoxic gas (21% O2) containing 5% CO2 and 74% N2 (pH 7.4). Each ring was connected to a model FT03 force transducer (Grass Instrument, Quincy, MA) for measurements of isometric tension. Recordings were made on a Grass polygraph or personal computer-based data acquisition program. Vascular rings were stretched and equilibrated for 60 min at a passive force of 1.5 g for the PA and 0.5 g for the PV. These resting tensions were determined to be optimal, on the basis of KCl length-tension responses performed with identically prepared vessels of similar dimensions. In some vessels, the endothelium was disrupted by gentle rubbing of the lumen with curved forceps. Effectiveness of the endothelium disruption was ascertained by functional responses to ACh.

Conducting vessel study protocol: constrictor responses. After a 1-h equilibration period, the contractile response of the vessel to the addition of 50 mM KCl was recorded. Rings that did not increase tension by at least 150 mg in response to KCl did not increase tension by at least 150 mg in response to KCl challenge were excluded from further study. After a 30-min recovery period, arteries and veins were preconstricted with the thromboxane mimetic U-46619 (0.001–0.1 μM).

Conducting vessel study protocol: dilator responses. Cumulative concentration-dependent responses to BK (0.0001–0.1 μM) in the presence or absence of l-NA, Indo, Glib, or CTX were measured in PA and PV rings preconstricted with U-46619. (For doses and molecular targets, see Table 1.) To avoid the confounding effect of tachyphylaxis, we used separate vascular rings to compare the BK-mediated relaxation

Table 1. List of chemicals, dosages, and targeted signaling pathways used

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dose</th>
<th>Targeted Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitro-l-arginine</td>
<td>0.1 mM</td>
<td>NOS inhibitor</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.01 mM</td>
<td>COX inhibitor</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.01 μM</td>
<td>K-ATP inhibitor</td>
</tr>
<tr>
<td>Charybdoxin</td>
<td>0.05 μM</td>
<td>K-Ca inhibitor</td>
</tr>
<tr>
<td>HOE-140</td>
<td>0.01 mM</td>
<td>B2 receptor antagonist</td>
</tr>
<tr>
<td>4-Aminopyridine</td>
<td>4 mM</td>
<td>K-DR inhibitor</td>
</tr>
<tr>
<td>Apamin</td>
<td>1 μM</td>
<td>K-Ca inhibitor</td>
</tr>
<tr>
<td>KCl</td>
<td>80 mM</td>
<td>Depolarizes membrane potential</td>
</tr>
<tr>
<td>CTX plus APA</td>
<td>0.05 μM, 1 μM</td>
<td>Inhibits EDHF-mediated dilations</td>
</tr>
<tr>
<td>Miconazole</td>
<td>30 μM</td>
<td>CYP450 epoxygenase inhibitor</td>
</tr>
<tr>
<td>17-Octadecyenoic acid</td>
<td>50 μM</td>
<td>CYP450 inhibitor</td>
</tr>
</tbody>
</table>

Chemicals are listed in order of their appearance in Figs. 1–10. None of these chemicals are cell-type specific and will inhibit the targeted pathway, if present, on endothelial, vascular smooth muscle (VSM) and adventitial cells. Known limitations regarding specificity or selectivity of the chemicals used include the following: nitro-l-arginine (l-NA), a nonselective nitric oxide synthase (NOS) inhibitor, will prevent NO synthesis from all NOS isoforms by competing with the NOS substrate arginine; indomethacin (Indo), a nonselective cyclooxygenase (COX) inhibitor, inhibits both COX-1 and COX-2 isoforms; glibenclamide (Glib), a selective inhibitor of ATP-sensitive potassium channels (K-ATP) (is present on both endothelium and VSM); charybdoxin (CTX), at the concentration used, selectively inhibits intermediate and large-conductance calcium-dependent potassium channels (K-Ca) on both endothelium and VSM; HOE-140, selective bradykinin 2 receptor antagonist, will block B2 receptors on VSM and endothelium; 4-aminopyridine (4-AP) is selective for voltage-gated (delayed rectifier) K+ channels (K-DR) on the VSM (endothelium lack K-DR channels); apamin (APA), selective inhibitor of small-conductance K-Ca is present on both endothelium and VSM; KCl nonselectively blocks dilations mediated by membrane hyperpolarization; CTX plus APA, when used together, block endothelium-derived hyperpolarizing factor (EDHF)-mediated dilations; miconazole is a cytochrome P-450 (CYP450) epoxygenase inhibitor, with numerous nonspecific effects reported; it binds to heme and inhibits enzymes involved in drug metabolism, steroidogenesis, and the synthesis of NO (53). Antimycotic agents, such as miconazole, can also alter intracellular calcium concentrations and ion channel activity in some cell types (53); 17-octadecyenoic acid (17-ODYA), a specific suicide substrate inhibitor of CYP450, will block the formation of 20-hydroxyicosatetraenoic acid and the epoxyeicosatrienoic acids (53).
responses under control conditions and in the presence of the inhibitors. Inhibitors were added to the bath after constriction to U-46619 and 15–30 min before addition of the lowest concentration of BK. At the end of each study, PA and PV were constricted with 120 mM KCl, followed by dilution with SNP (0.01 mM) and/or papaverine (0.1 mM) and calcium-free buffer to verify functional VSM.

Isolation of PRA for measurement of pressurized lumen diameter. Under a dissecting microscope, piglet PRA were identified, excised, and trimmed of excess connective tissue. Isolated vessels were transferred to an arteriograph (Living Systems Instrumentation, Burlington, VT) containing two glass microcannula and inlet and outlet ports connected to a circulation reservoir for superfusion of the adventitial side of the vessel. PRA were studied in a modified Krebs-Henseleit buffer with the following composition (in mM): 118 NaCl, 25 NaHCO3, 4.8 KCl, 1.2 MgSO4·7 H2O, 1.2 KH2PO4, 11 dextrose, and 2.0 CaCl2·2 H2O equilibrated with 21% O2, 5% CO2, balance N2 to maintain pH 7.4. PRA, measuring 100–300 μm in diameter, were cannulated at one end, secured with a single strand of thread, gently flushed free of blood, and cannulated at the distal end. All side branches (typically 1–3 per vessel) were tied with a single fiber of 10-0 multifilament braided thread. A pressure servo-system connected to the proximal cannula maintained intraluminal pressure at 15–18 mmHg. Prewarmed (37°C) buffer was circulated through the vessel chamber at a rate of 30 ml/min; the same gas mixture flowed over the vessel chamber, housed under the superfusion gas cover. The arteriograph was set in a television monitor, and continuous measurement of the lumen diameter (LD) was made with a video dimension analysis system (Living Systems Instrumentation).

**PRA study protocol: constrictor responses.** After a 30-min equilibration period with stable LD, PRA were constricted by addition of 50 mM KCl, followed by addition of ACh (0.1 μM) or A-23187 (0.1 μM) to verify intact VSM function and endothelium-dependent relaxation, respectively. After another 30-min equilibration period at baseline LD, PRA were constricted by cumulative addition of U-46619 (0.001–1.0 μM) to achieve a 40–60% decrease in LD.

**PRA study protocol: dilator responses.** Dilator responses to increasing concentrations (0.0001–1.0 μM) of BK were determined in control (untreated) PRA and in PRA treated with L-NA, Indo, CTX, APA, 4-AP, or Glib for 15 min. To avoid spurious results due to tachyphylaxis, we used separate vessels to evaluate the responses to BK under control conditions and in the presence of inhibitors. In some PRA, we mechanically disrupted the endothelium by sliding the vessels over a strand of human hair (48). In other vessels, we destroyed the endothelium by opening the stopcock on the outflow cannula and perfusing 2–10 ml of air, followed by a 10-min buffer perfusion to flush out the separated endothelium layer (43). Only endothelium-disrupted PRA that failed to dilate in response to ACh or A-23187 were studied. At the end of each study protocol, both endothelium-intact and -disrupted PRA were constricted with 120 mM KCl, followed by dilution with SNP (0.01 mM) and/or papaverine (0.1 mM) and calcium-free buffer to verify functional VSM.

**Statistical analysis.** BK concentration-response profiles are graphically depicted as means ± SE. Except where indicated, n refers to the number of piglets studied. Relaxation responses in conducting vessels are expressed as percent relaxation of the U-46619-induced tension. In PRA, responses are expressed as percent dilation from the U-46619-induced change in LD. The concentration-response curves were fitted to a sigmoidal relation that yielded a value for percent maximal relaxation (Rmax) and the concentration that produced half-maximal response (log EC50) with GraphPad Prism 3 (San Diego, CA). These values were analyzed by one-way ANOVA and a Tukey’s post hoc multiple comparison test. Using SPSS 10.0, we analyzed the concentration-dependent relaxation responses in the various study groups by general linear modeling with repeated measures followed by a Scheffe’s post hoc multiple comparison test at the 0.05 level of significance.

**RESULTS**

**Constrictor responses to U-46619.** Time control studies demonstrated that cumulative addition of the thromboxane mimetic U-46619 (0.001–1 μM) to both endothelium-intact and -disrupted pulmonary conducting vessels caused a sustained and stable contractile response. Mean U-46619-induced constriction was 645 ± 54 mg in PA rings and 1,720 ± 142 mg in PV rings. In endothelium-intact and -disrupted PRA, U-46619 (0.001–1.0 μM) also caused a stable and sustained constriction, resulting in 54.4 ± 2.1% decrease in LD.

**Dilator responses to BK.** BK-induced concentration-dependent relaxations in PA rings, PV rings, and pressurized PRA. The mean Rmax and log EC50 values with 95% confidence intervals for BK-induced relaxation in PA, PV, and PRA are shown in Table 2. The Rmax to BK was similar in large PA rings and PRA; Rmax was significantly smaller in PV rings than in either the conducting or resistance arteries. Sensitivity to BK, as assessed by the log EC50 values, was nearly one log molar concentration lower in pressurized PRA than in PA and PV rings (Table 2).

**Investigations of BK-mediated relaxation in conducting vessels.** Removal of the endothelium or inhibition of NOS with L-NA abolished the BK-mediated relaxation in PA, PV, and PRA (Table 2). The concentration-dependent relaxation responses to BK were not significantly inhibited by Indo, Glib, or CTX in PA (Fig. 1) or PV rings (Fig. 2).

**Investigations of BK-mediated dilation in resistance vessels.** BK-induced dilation was completely blocked in the presence of HOE-140, demonstrating that the observed relaxation was specific to BK and mediated via activation of the B2 receptor (Fig. 3).

<table>
<thead>
<tr>
<th>Vessel Type</th>
<th>Rmax, %</th>
<th>Log EC50, M</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>74.0 (69.7 to 78.2)</td>
<td>−8.5 (−8.6 to −8.4)</td>
</tr>
<tr>
<td>PV</td>
<td>51.7 (45.0 to 58.4) *</td>
<td>−8.4 (−8.6 to −8.1)</td>
</tr>
<tr>
<td>PRA</td>
<td>76.5 (72.5 to 80.5) †</td>
<td>−7.8 (−7.9 to −7.7)</td>
</tr>
</tbody>
</table>

Values are expressed as means (±95% confidence intervals). Rmax, maximal relaxation; log EC50, concentration that produced half-maximal response; PA, pulmonary artery; PV, pulmonary vein; PRA, pulmonary resistance artery. * Different from PA and PRA (P < 0.01); † different from PA and PV (P < 0.05).
Role of nitric oxide, prostaglandins, and $K^+$ channel activation. Nitric oxide synthase (NOS) inhibition with L-NA blocked approximately one-third of the dilation response to BK in PRA (Fig. 3). Indo alone (Fig. 4) or in combination with L-NA (not shown) had no effect. Likewise, Glib, a selective inhibitor of ATP-sensitive potassium channels (K-ATP), had no effect on BK-induced dilation (Fig. 4). In contrast, 4-AP, an inhibitor of delayed rectifier potassium channels (K-DR), partially inhibited the response (Fig. 4). CTX, an inhibitor of large- and intermediate-conductance calcium-dependent potassium channels (K-Ca), partially inhibited the relaxation response to BK (Fig. 5). No statistical differences were found between the BK concentration-response curves in control PRA and those treated with APA, an inhibitor of small-conductance K-Ca (Fig. 5). The combination of L-NA plus CTX did not cause a combination with L-NA (not shown) had no effect. Likewise, Glib, a selective inhibitor of ATP-sensitive potassium channels (K-ATP), had no effect on BK-induced dilation (Fig. 4). In contrast, 4-AP, an inhibitor of delayed rectifier potassium channels (K-DR), partially inhibited the response (Fig. 4). CTX, an inhibitor of large- and intermediate-conductance calcium-dependent potassium channels (K-Ca), partially inhibited the relaxation response to BK (Fig. 5). No statistical differences were found between the BK concentration-response curves in control PRA and those treated with APA, an inhibitor of small-conductance K-Ca (Fig. 5). The combination of L-NA plus CTX did not cause a
further decrease in dilation, compared with either drug alone (Fig. 5).

**Role of endothelium-derived hyperpolarizing factor.** To determine whether BK stimulates release of endothelium-derived hyperpolarizing factor (EDHF) and whether EDHF release is responsible for the L-NA-insensitive component of the BK-induced relaxation response, we constricted PRA with a depolarizing concentration of KCl rather than with U-46619. In KCl-constricted PRA, BK induced a concentration-dependent dilation in the absence of NOS inhibition (Fig. 6). However, in the presence of L-NA, BK-induced dilation was completely abrogated (Fig. 6).

EDHF-induced relaxations and hyperpolarizations are inhibited by the combination of CTX and APA but not by either drug alone (6, 9, 14, 16, 17, 50). Accordingly, we examined the BK-induced dilation response in PRA in the presence of CTX plus APA. Indo was added to the arteriograph in all studies to eliminate the contribution of dilator prostanoids. Joint application of CTX and APA significantly inhibited BK-induced dilation (Fig. 7). Nearly identical inhibition of the BK-induced relaxation response was observed with the combination of L-NA plus APA (Fig. 7). Furthermore, when we combined inhibition of NOS with L-NA, plus inhibition of EDHF with CTX plus APA, the BK-induced dilation was completely abolished (Fig. 7).

**Role of cytochrome P-450 metabolites of arachidonic acid.** Because in some vessel types, EDHF is a cytochrome P-450 metabolite of arachidonic acid (22, 29), we examined the dilation response of PRA to BK in the presence of the cytochrome P-450 monooxygenase inhibitor miconazole and the suicide substrate inhibitor of cytochrome P-450 epoxygenase 17-ODYA. All studies with cytochrome P-450 inhibitors were performed in the presence of Indo. Neither miconazole (30 μM) nor 17-ODYA (50 μM) alone significantly inhibited BK-mediated dilation in PRA exposed to the combination of L-NA plus one of the cytochrome P-450 inhibitors (miconazole or 17-ODYA) was not statistically different than the inhibition caused by L-NA alone (Fig. 8).

**Role of endothelium-independent dilation.** To investigate the endothelium dependency of the dilation response, we conducted studies in PRA perfused with a bolus of air to mechanically disrupt the endothelium. There were no differences in baseline LD (Fig. 9) or LD after constriction to KCl (50 mM, not shown) or U-46619 (1.0 μM; Fig. 9) between endothelium-intact and endothelium-disrupted PRA.
and disrupted PRA. Endothelium-intact PRA potently dilate when stimulated with ACh or A-23187 (Fig. 9). In contrast, endothelium-disrupted PRA constrict in response to ACh (0.1 M), a receptor-mediated endothelium-dependent dilator, or to A-23187 (0.1 M), an endothelium-dependent but receptor-independent dilator (Fig. 9). As shown in Fig. 10, endothelium-disrupted PRA dilated in response to increasing concentrations of BK. Identical results were obtained with six PRA mechanically stripped of the endothelium by sliding the vessel over a strand of human hair (data not shown). The B2 receptor antagonist HOE-140 prevented the BK-mediated dilation in endothelium-disrupted PRA (n = 2, data not shown). To determine whether BK directly activates VSM K-Ca channels to mediate endothelium-independent dilation, we exposed endothelium-disrupted PRA for 30 min to CTX alone or CTX plus APA. As shown in Fig. 10, BK-induced dilation in endothelium-disrupted PRA is nearly abolished by CTX or by the combined addition of CTX plus APA.

DISCUSSION

Treatment of persistent pulmonary hypertension of the newborn (PPHN) is impeded by an incomplete understanding of the basic mechanisms regulating vasoreactivity in the perinatal pulmonary circulation. Elucidation of the signaling mechanisms responsible for vasoactive responses at the level of the pulmonary resistance vasculature is essential to our understanding of fetal-to-neonatal transitional physiology and the failure of circulatory adaptation that characterizes the clinical syndrome of PPHN. Until recently, most investigations of pulmonary vascular responses have been conducted in large conducting vessels. The technology is now available to study vasoreactivity in pressurized PRA. In contrast to measurement of isometric tension in vascular rings, cannulated resistance arteries retain their in vivo shape, are subjected to a transmural wall pressure, and have an endothelium that is unperturbed by wires. Resistance arteries are active participants in the physiological regulation of blood pressure and blood flow distribution and thus may yield information more relevant to in vivo responses (28, 41, 63).

BK is one of the most potent vasodilators known. BK causes pulmonary vasodilation in vivo (24), in isolated perfused lung preparations (49), and in isolated pulmonary vessels in vitro (3, 4, 10, 46, 64). In the perinatal pulmonary circulation, the effects of BK vary considerably depending on the species, age, and segmental origin of the blood vessel studied (3, 4).

These studies are the first to explore the effects of BK in cannulated, pressurized PRA. Our findings illus-
trate that BK causes marked dilation in all segments of the newborn pig pulmonary circulation. Our studies also demonstrate segmental differences in the signaling mechanisms contributing to BK-mediated relaxation, with multiple signaling mechanisms that are uniquely functional at the level of the resistance circulation.

Role of the endothelium. A novel finding of this study is the identification of an endothelium-independent dilator pathway stimulated by BK in resistance but not in conducting, vessels. It has generally been reported that BK is an endothelium-dependent vasodilator (10, 30, 40). Indeed, our own findings in conducting vessels support that conclusion (Figs. 1 and 2). In contrast, endothelium-disrupted PRA retain their ability to dilate in response to BK (Fig. 10).

Although it is tempting to speculate that failure to completely remove all endothelial cells from the PRA preparation accounts for this finding, we believe this to be unlikely as we obtained identical results with two different mechanical methods to disrupt the endothelium (hair and air) and we included in our analysis only PRA that failed to dilate in response to ACh or A-23187. ACh has long been regarded as the benchmark for demonstrating the functional integrity of the endothelium in vascular preparations. Endothelium-intact piglet PRA consistently dilate in response to ACh (mean dilation 68.5 ± 2.8%) and A-23187 (mean dilation 75.9 ± 3.1%). In contrast, endothelium-disrupted PRA develop a constrictor response to both endothelium-dependent vasodilators (Fig. 9). As A-23187 is a receptor-independent vasodilator, these findings demonstrate that the air bolus did not simply damage the muscarinic receptors but, in fact, disrupted the functional integrity of the endothelium.

In PRA lacking a functional endothelium, stimulation with BK exposes an endothelium-independent dilator mechanism that is mediated via activation of the B2 receptor subtype, as it is inhibited by HOE-140 in both endothelium-intact and -disrupted PRA. The cellular location(s) of the B2 receptors responsible for the dilation response in intact or disrupted PRA cannot be determined from these studies; B2 receptors are expressed on endothelial cells, VSM cells, and adventitial fibroblasts (11, 62). In endothelium-disrupted PRA, the downstream dilator mechanism involves the activation of K-Ca, as it is inhibited by CTX (Fig. 10) and abolished in KCl-constricted, endothelium-disrupted PRA (data not shown). This endothelium-independent dilator mechanism is obscured in endothelium-intact PRA and would not be recognized in studies that failed to include investigations in endothelium-disrupted vessels.

These are not the first studies to report endothelium-independent vascular responses to BK. Theis et al. (59) found that BK dose dependently relaxed endothelium-denuded PRA rings in the lamb. Likewise, BK-induced relaxation in superior mesenteric arteries from cats and rabbits is unaffected by removal of the endothelium (10, 23, 53). In hamster superior mesenteric artery, BK induces hyperpolarization in both endothelium-intact and -disrupted vessels (58) that were mediated via prostaglandin release and activation of K+ATP. Thus, although endothelium-independent vasodilation is not unprecedented, there appears to be species and regional heterogeneity in the signaling mechanisms mediating both endothelium-dependent and -independent vascular responses to BK.

Role of NO. Another important finding of this study is a greater dependence of conducting vs. resistance vessels on endothelium-dependent NO-mediated dilation. In contrast to large conducting vessels where L-NA abolished the response (Figs. 1 and 2), in PRA only one-third of the BK-induced dilation was inhibited by L-NA (Fig. 3). The failure of NOS inhibition to prevent bradykinin-induced vasodilation has been described in resistance vessels from other circulatory beds and other species (45, 61). Particularly in the coronary microcirculation of the dog (45), pig (61), and human (34, 35, 44), NO-independent mechanisms play a dominant role in the dilation response to BK.

Role of prostaglandins. We were unable to identify a cyclooxygenase (COX)-dependent component of the BK dilation response in large conducting rings or PRA (Fig. 4). This is similar to findings in mature pigs (20), newborn guinea pigs (13), and adult dogs and cats (10, 36) but differs from studies in fetal lamb lungs where BK causes pulmonary vasodilation in part by release of dilator prostanoids (24). In superior mesenteric arteries from cats and rabbits, BK-induced dilation is completely blocked by COX inhibition (10, 23, 53). In some species, the importance of dilator prostaglandins abates with age beyond the immediate postnatal period (46, 64).}

Role of K+ channel activation. Our studies clearly demonstrate that K+ channel activation plays an important role in the dilation response to BK only in resistance-level arteries and that multiple K+ channel subtypes are involved. Inhibition of K-DR with 4-AP blunted the dilation response to BK in PRA (Fig. 4). To our knowledge, this is the first report of a role for a 4-AP-sensitive channel in the response of resistance vessels to BK, although a role for these channels has been reported in the dilation of coronary resistance arteries to ACh (14). Interestingly, Archer and colleagues (1) have shown differences in the distribution and prevalence of VSM cell K+, with more K-Ca in conduit arteries and more K-DR channels in resistance arteries. Reeve et al. (52) have shown a maturational shift in the expression of K+ channels regulating resting pulmonary vascular tone from K-Ca predominance in the fetus to K-DR in the adult.

Our studies also indicate that in PRA, but not in large conducting vessels, BK elicits relaxation by a mechanism that is partially dependent on activation of K-Ca. CTX alone (Fig. 5) or administered jointly with APA (Fig. 7) significantly blunts the dilation response. In endothelium-intact PRA, BK may directly or indirectly activate K-Ca by increasing intracellular Ca2+ levels and/or by releasing of one or more endothelium-derived vasodilators. NO has been shown to activate K+ channels, either directly (5) or via a G kinase-
mediated phosphorylation event (8). Data that support this mechanism of NO-mediated dilation include the similar degree of inhibition induced by l-NA and by CTX and the failure of l-NA + CTX to have additive effects (Fig. 5). However, it is likely that NO can also elicit relaxation by a mechanism independent of K channel activation, because, as shown in Fig. 6, preventing membrane hyperpolarization by constricting vessels with KCl does not prevent the dilation response to BK, as long as the NO pathway is unperturbed.

Role of EDHF. Although the chemical identity of EDHF remains elusive, the production of an endothelium-derived relaxing factor that produces VSM hyperpolarization has been demonstrated in various systemic vascular beds (9, 16, 21, 27). EDHF-mediated relaxations are inhibited by membrane depolarization with KCl and by the combination of CTX and APA (6) but are insensitive to inhibitors of NOS, COX, and K-ATP (15, 21). It is likely that there is more than one EDHF, varying with the species and vascular bed studied (12, 16, 22, 51). In some vascular beds, EDHF is reported to have greater sensitivity in resistance than in conducting vessels (6, 27, 32, 42, 45, 47). NO has been shown to exert feedback inhibition on EDHF-mediated dilations and on the cytochrome P-450 pathway (61). Thus the contribution of EDHF is often best observed under conditions of NOS inhibition.

In piglet PRA, CTX, combined with APA (Fig. 7), and membrane depolarization with KCl (Fig. 6) abolish the l-NA-insensitive component of the response, findings consistent with a role for EDHF. However, inhibitors of ion channels, such as CTX and APA, are not cell-type specific. Both drugs can inhibit K-Ca on endothelial and VSM cells. Thus there are several possible interpretations of the effects of CTX and APA in PRA. CTX and APA could inhibit NOS-independent dilation by inhibiting EDHF. Alternatively, CTX and APA could directly inhibit large-, intermediate-, and/or small-conductance K-Ca on endothelial cells and/or VSM cells, independently of the release of an EDHF. CTX-sensitive large-conductance and IK channels are present on endothelial cells and have been shown to be activated by BK (25, 33). In endothelium-disrupted PRA, CTX must exert its effect by inhibiting K-Ca activation on the VSM. Care must be taken when interpreting results on the basis of application of K+ channel blockers that can act on the endothelial cell or VSM cell, or both.

Role of cytochrome P-450 metabolites. Cytochrome P-450 metabolites of arachidonic acid, in particular the epoxyeicosatrienoic acids, have been shown to mediate vasodilation and have been identified as possible EDHFs (22, 29). Using two different cytochrome P-450 inhibitors, miconazole and 17-ODYA, our studies do not support a significant role for a cytochrome P-450-derived EDHF in the newborn pig pulmonary microcirculation. When used alone, neither miconazole nor 17-ODYA significantly inhibits BK-induced dilation (Fig. 8). Furthermore, the inhibition of the BK-induced dilation response in the presence of l-NA plus miconazole or l-NA plus 17-ODYA was not statistically different than the inhibition caused by l-NA alone (Fig. 8). Boels et al. (4) reported inhibition of the NOS-independent dilation to BK with SKF525a, another inhibitor of the cytochrome P-450 pathway. It is important to note that many cytochrome P-450 inhibitors have nonspecific effects, including alterations in intracellular calcium concentrations and ion channel activity, including inhibition of K-Ca (18, 33, 54).

In summary, BK-induced relaxation is mediated by endothelium-derived NO in large conducting PA and PV. In PRA, BK elicits dilation by multiple signaling pathways. Release of NO and EDHF mediates the response in endothelium-intact PRA. Activation of K+ channels, including K-DR and K-Ca, also contribute to BK-mediated dilation. Under conditions of endothelial disruption, dilation is preserved by BK-mediated activation of VSM K+ channels.

The biological implications of multiple and redundant BK-mediated signaling pathways in the perinatal pulmonary circulation are speculative. Prolonged exposure to the hypoxic in utero environment and high PA pressures throughout fetal development may result in diminished NO-mediated responses or a state of relative endothelial dysfunction in conducting PA. At the level of the pulmonary resistance vasculature, alternative dilator mechanisms may be essential to ensure successful pulmonary circulatory adaptation to extrauterine life. BK is unique among dilator stimuli. Unlike ACh, a dilator that is unlikely to play a functional role in the perinatal pulmonary circulation, BK is thought to be a mediator of transitional pulmonary adaptation at birth. Our results suggest that this peptide is well suited to this role, as BK evokes a unique endothelium-independent dilator mechanism in PRA of the newborn that may augment or substitute for complementary endothelium-dependent dilator pathways to assure a smooth transition to postnatal life.

The authors gratefully acknowledge the technical and intellectual contributions of Dr. Cathy Davison to the pilot study for this project. Supported by the National Heart, Lung, and Blood Institute Grant HL-62489, March of Dimes Research Grant 6-FY96-0703, and by American Lung Association Research Grant RG-080-N (to J. L. Aschner).

REFERENCES

endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. Proc Natl Acad Sci USA 97: 9747–9752, 2000.


